METHOD

This invention relates to use of polymorphisms in human OATP-C in statin therapy because they are associated with an effect on statin pharmacokinetics (PK) in humans, especially rosuvastatin pharmacokinetics. The invention also relates to the use of OATP-C polymorphisms in predicting the efficacy and safety of statins, whose uptake in to the liver is mediated by OATP-C, especially rosuvastatin.

The OATP-C gene (sometimes called LST1, OAPT2, SLCO1B1, SLC21A6 or OATP1B1) has been cloned by four different groups, annotated and published as EMBL accession numbers AB026257 (OATP-C, 2452bp), AF205071(OATP2, 2830, SEQ ID herein), AJ132573(OATP2, 2778), and AF060500 (LST-1). Konig (2000) J Biol Chem 275, 23161-23168 describes the genomic organisation of OATP 1, 2 and 8. International patent application WO 00/08157 describes human anion transporter genes and some polymorphisms.

Na+-independent organic anion transporting polypeptide (OATP) C gene is a member of the OATP supergene family involved in multifunctional transport of organic anions. OATP-C transports a diverse range of molecules e.g. the organic anion taurocholate, conjugated steroids: DHEAS, estradiol 17β-D-glucoronide and estrone-3-sulfate, eicosanoids: PGE_2, thromboxane B_2, leukotriene C_4, and E_4, and thyroid hormones T4 and T3. OATP-C has also been shown to be involved in the transport of xenobiotics, and drugs involved in lipid lowering *e.g.* statins. Statins are a class of drugs which inhibit 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA). They are an important therapy for patients with atherosclerotic vascular diseases and are generally well tolerated, although some rare adverse events have been noted in all marketed statins. Pharmacokinetic differences between statins have been associated with differences in benefit-risk ratio (Igel (2002) J Clin Pharmacol 42: 835-45).

It is generally recommended that in order to gain maximum benefit-risk ratio from statin therapy, the dose prescribed should be individualized according to goal of therapy and response. For example, the recommended usual starting dose of rosuvastatin is 10 mg once daily in patients with hypercholesterolemia and mixed dyslipidemia, although a 5-mg dose is also available. For patients with marked hypercholesterolemia (LDL-C >190 mg/dL) and aggressive lipid targets, a 20-mg starting dose may be considered. The 40-mg dose should be reserved for those patients who have not achieved goal LDL-C at 20 mg. After initiation and/or upon titration of rosuvastatin, lipid levels should be analyzed within 2 to 4 weeks and dosage adjusted accordingly.

Pravastatin is actively transported from the circulation to the liver via the OATP-C transporter (Hsiang, B. Journal of Biological Chemistry. 274(52), 37161-37168. 1999). Rosuvastatin was shown to be a substrate for OATP-C *in vitro* (Brown (2001) Atherosclerosis Supplements **2**, pg 90, poster abstract P174). Numerous polymorphisms in OATP-C have been reported in the literature and SNP databases. Identification of SNPs in OATP-C was reported in EP1186672. Polymorphism in OATP-C has been reported by Tamai *et al* (2000), BBRC, **273**, 251-60 and reviewed by Tirona (2002) Adv Drug Deliery Reviews 54:1343-52. Tirona showed that some OATP-C polymorphisms, including the V174A variant, were associated with reduced transport of endogenous substrates *in vitro*. Using a different cell system, Nozawa et al (2001) J Pharmacol Exp Ther, **302**, 804-13, found that the V174A (OATPC*5) variant did not affect substrate transport. Tirona stated that the *in vivo* relevance of OATP-C polymorphisms remained to be determined.

Niemi et al (July 2004) Pharmacogenetics 14(7), 429-440 describes SNPs in OATPC, including promoter SNPs. The authors report a significant correlation between pravastatin PK (pharmacokinetics) and a haplotype containing a promoter SNP in a cohort of healthy volunteers.

Nishizato (2003) Clin Pharmacol Ther **73**:554-65 published *in vivo* data showing that the OATPC*15 allele, containing both the N130D and the V174A polymorphisms, had an effect on the pharmacokinetics of pravastatin in Japanese healthy volunteers. Nishizato did not report the effect of the OATP-C*5 allele, which has not been detected in the Japanese population to date, and stated that large clinical studies are needed to investigate this effect. There have been no pharmacokinetic studies in patients taking pravastatin. There have been no pharmacogenetic studies on healthy volunteers or patients taking rosuvastatin. Hence, the observations of Nishizato et al performed in Japanese healthy volunteers are not predictive of the PK profiles of patients or of other populations, or of the PK profiles of other statins which may differ in the affinity for different transporters.

Population PK modelling analyses, using data collected by AstraZeneca in the rosuvastatin clinical development programme, confirm that healthy volunteers and patients differ with respect to their distribution of rosuvastatin. Patients receiving statins for lipid lowering may be on other drugs transported by OATP-C and drug-drug interactions may affect the PK profile of statins (Int J Clin Pharmacol Ther (2002), 40, 439-50). Patients prescribed statins may also have other liver and kidney complications affecting the distribution and excretion of statins. There is an entire chapter in the textbook Clinical

Pharmacokinetics (Chapter 16, pages 248-266 in 3rd edition 1995, Rowland & Tozer, published by Williams & Wilkins) which begins:

"Disease is a major source of variability in drug response. For many diseases this is due primarily to differences in pharmacokinetics..."

Hence there is a need to identify which polymorphisms in OATP-C have an effect on *in vivo* pharmacokinetics of rosuvastatin and other statins in patients with vascular disease or a predisposition thereto. Our invention is based on the discovery that the V174A polymorphism and/or a polymorphism in linkage disequilibrium therewith has a statistically significant effect on statin pharmacokinetics in patients. The V174A polymorphism may affect the response to statins, especially rosuvastatin.

According to one aspect of the invention there is provided a method of diagnosis comprising:

(a) providing a biological sample from a human identified as being in need of treatment with a therapeutic agent that is transported by OATP-C, wherein the sample comprises a nucleic acid encoding OATP-C;

(b) testing the nucleic acid for the presence, on at least one allele, of either

(i) a codon encoding alanine at the position corresponding to position 174 of SEQ ID NO:1, or

(ii) an allele of a polymorphism in linkage disequilibrium with (i); and

(c) if either (i) or (ii) is found in at least one allele, diagnosing the human as likely to have reduced ability to transport the therapeutic agent into cells.

Preferably the polymorphism of (b)(ii) is -26A>G, -118A>C, -309T>C, -878A>G, -903C>T, -1054G>T, -1215T>A, or -1558T>C, all of SEQ ID NO:2; or T2122G, C2158T, A2525C, or G2651A, all of SEQ ID NO:3.

More preferably the polymorphism of (b)(ii) is -118A>C or –1558T>C of SEQ ID NO:2. Alleles of polymorphisms at -118 and -1558 are in significant linkage disequilibrium with the alanine allele at position 174 of SEQ ID NO:1 (p=0.009 and 0.025respectively, analysed by the ASSOCIATE program, see Ott J (1999) Analysis of human genetic linkage, 3rd edition. Johns Hopkins University Press, Baltimore).

Most preferably the polymorphism of (b)(ii) is -118A>C of SEQ ID NO:2; Example 2 hereinbelow describes the functional effect of this polymorphism.

For any variant position, for example –26A>G, this indicates that at position 26 A is replaced by G. This can be tested by either detecting G at that position or by detecting that there is not an A at that position. Similar considerations apply to any variant position.

Preferably the therapeutic agent is a statin, the human is being treated with one dose level of statin and step (c) further comprises diagnosing the human as suitable for titration to another higher statin dose level comprising monitoring for a decrease in benefit-risk ratio resulting from the reduced ability to transport the statin into cells.

More preferably the therapeutic agent is rosuvastatin.

In another embodiment the therapeutic agent is one of atorvastatin, cerivastatin, fluvastatin, pravastatin, or simvastatin.

Preferably the human is being treated with at least 5 mg of a rosuvastatin daily. More preferably the human is being treated with at least 10 mg of a rosuvastatin daily. More preferably the human is being treated with at least 20 mg of a rosuvastatin daily. More preferably the human is being treated with at least 40 mg of a rosuvastatin daily.

According to another aspect of the invention there is provided a method of diagnosis comprising:

(a) providing a biological sample from a human identified as being in need of treatment with a therapeutic agent that is transported into cells by OATP-C, wherein the sample comprises an OATP-C polypeptide;

(b) determining whether the amino acid of OATP-C corresponding to position 174 of SEQ ID NO:1 is a valine; and

(c) if the amino acid is not a valine, diagnosing the human as likely to have a reduced ability to transport the therapeutic agent into cells.

Preferably the therapeutic agent is a statin, the human is being treated with one dose level of statin and step (c) further comprises diagnosing the human as suitable for titration to another higher statin dose level comprising monitoring for a decrease in benefit-risk ratio resulting from the reduced ability to transport the statin into cells.

Preferably the method further comprises measurement of the level of OATPC polypeptide with valine and/or alanine at position 174 whereby to determine the presence or absence of -118A>C polymorphism in OATP-C nucleic acid.

Preferably the method is one further comprising measuring the level of OATP-C polypeptide for presence or absence of OATP-C *15 allele whereby to determine the presence or absence of -118A>C polymorphism in OATP-C nucleic acid.

Without wishing to be bound by theoretical considerations, the level of protein expression of each of the different allelic forms of OATPC may be determined where the level of expression of the Ala174variant is increased due to enhanced promoter activity in the presence of the linked -118C promoter variant. For example, by determining the ratio of Val174 : Ala174 protein isoforms, where the Ala 174 reduced function protein is present in excess of the Val 174 normal function transporter in subjects heterozygoyte at position 174 in the amino acid sequence. In another example, by determing the absolute level of OATPC expression in Ala174 homozygote subjects, and comparing the absolute expression level to that of the population mean for subjects homozygote for the Val174 variant where the relative expression of the Ala 174 allele is increased in the presence of the -118C linked promoter allele, as compared to Ala 174 alleles linked to the -118A promoter variant. In another example, by determing the absolute level of OATPC expression in Ala174 homozygote subjects, and comparing the absolute expression level to that of the population mean, Ala174 homozygote subjects, with the highest levels of OATPC expression, may be predicted to have one or more copies of the -118C promoter variant through the increased transcription activity of the minor allelic form of the OATPC promoter.

Preferably the amino acid at position 174 is determined to be alanine. More preferably the therapeutic agent is rosuvastatin.

In another embodiment the therapeutic agent is one of atorvastatin, cerivastatin, fluvastatin, pravastatin, or simvastatin.

Preferably the human is being treated with at least 5 mg of a rosuvastatin daily. More preferably the human is being treated with at least 10 mg of a rosuvastatin daily. More preferably the human is being treated with at least 20 mg of a rosuvastatin daily. More preferably the human is being treated with at least 40 mg of a rosuvastatin daily.

According to one aspect of the present invention there is provided an in vitro diagnostic method to identify a patient potentially requiring a statin dose level above the minimum recommended dose level or to identify a patient in which titration to a statin dose level above the minimum recommended dose level should be monitored in which the method comprises testing a biological sample from the patient for presence of alanine at position 174 of OATP-C polypeptide and/or a polymorphism in linkage disequilibrium therewith.

The biological sample is conveniently a sample of blood, bronchoalveolar lavage fluid, sputum, liver or other body fluid or tissue obtained from an individual. It will be appreciated that the test sample may equally be a nucleic acid sequence corresponding to the

sequence in the test sample, that is to say that all or a part of the region in the sample nucleic acid may firstly be amplified using any convenient technique e.g. PCR, before analysis of allelic variation. Preferably the patient is tested for presence of alanine at position 174 either through analysis of polypeptide directly or through analysis of genetic material encoding the polypeptide. As patients carry 2 copies of the OATPC gene they may be homozygous or heterozygous genotype. Polymorphisms in linkage disequilibrium with alanine at 174 may be tested as an alternative to determining the presence of alanine at 174 directly.

It will be apparent to the person skilled in the art that there are a large number of analytical procedures which may be used to detect the presence or absence of variant nucleotides at one or more polymorphic positions of the invention. In general, the detection of allelic variation requires a mutation discrimination technique, optionally an amplification reaction and optionally a signal generation system. Table 1 lists a number of mutation detection techniques, some based on the PCR. These may be used in combination with a number of signal generation systems, a selection of which is listed in Table 2. Further amplification techniques are listed in Table 3. Many current methods for the detection of allelic variation are reviewed by Nollau *et al.*, Clin. Chem. 43, 1114-1120, 1997; and in standard textbooks, for example "Laboratory Protocols for Mutation Detection", Ed. by U. Landegren, Oxford University Press, 1996 and "PCR", 2nd Edition by Newton & Graham, BIOS Scientific Publishers Limited, 1997.

Abbreviations:

ALEX™	Amplification refractory mutation system linear extension
APEX	Arrayed primer extension
ARMS™	Amplification refractory mutation system
b-DNA	Branched DNA
bp	base pair
CMC	Chemical mismatch cleavage
COPS	Competitive oligonucleotide priming system
DGGE	Denaturing gradient gel electrophoresis
FRET	Fluorescence resonance energy transfer
LCR	Ligase chain reaction
MASDA	Multiple allele specific diagnostic assay
NASBA	Nucleic acid sequence based amplification
OLA	Oligonucleotide ligation assay

PCR	Polymerase chain reaction
PTT	Protein truncation test
RFLP	Restriction fragment length polymorphism
SDA	Strand displacement amplification
SNP	Single nucleotide polymorphism
SSCP	Single-strand conformation polymorphism analysis
SSR	Self sustained replication
TGGE	Temperature gradient gel electrophoresis

Table 1 - Mutation Detection Techniques

General: DNA sequencing, Sequencing by hybridisation

Scanning: PTT*, SSCP, DGGE, TGGE, Cleavase, Heteroduplex analysis, CMC, Enzymatic mismatch cleavage

* Note: not useful for detection of promoter polymorphisms.

Hybridisation Based

Solid phase hybridisation: Dot blots, MASDA, Reverse dot blots, Oligonucleotide arrays (DNA Chips)

Solution phase hybridisation: Taqman™ - US-5210015 & US-5487972 (Hoffmann-La Roche), Molecular Beacons - Tyagi *et al* (1996), Nature Biotechnology, **14**, 303; WO 95/13399 (Public Health Inst., New York)

Extension Based: ARMS™, ALEX™ - European Patent No. EP 332435 B1 (Zeneca Limited), COPS - Gibbs *et al* (1989), Nucleic Acids Research, **17**, 2347.

Incorporation Based: Mini-sequencing, APEX

Restriction Enzyme Based: RFLP, Restriction site generating PCR

Ligation Based: OLA

Other: Invader assay

Table 2 - Signal Generation or Detection Systems

Fluorescence: FRET, Fluorescence quenching, Fluorescence polarisation - United Kingdom Patent No. 2228998 (Zeneca Limited)

Other: Chemiluminescence, Electrochemiluminescence, Raman, Radioactivity, Colorimetric, Hybridisation protection assay, Mass spectrometry

Table 3 - Further Amplification Methods

SSR, NASBA, LCR, SDA, b-DNA

Table 4- Protein variation detection methods

Immunoassay

Immunohistology

Peptide sequencing

Preferred mutation detection techniques include ARMS™, ALEX™, COPS, Taqman, Molecular Beacons, RFLP, and restriction site based PCR and FRET techniques.

Immunoassay techniques are known in the art e.g. A Practical Guide to ELISA by D M Kemeny, Pergamon Press 1991; Principles and Practice of Immunoassay, 2nd edition, C P Price & D J Newman, 1997, published by Stockton Press in USA & Canada and by Macmillan Reference in the United Kingdom. Histological techniques are described in Theory and Practice of Histological Techniques by J D Bancroft & A Stevens, 4th Edition, Churchill Livingstone,1996. Protein sequencing is described in Laboratory Techniques in Biochemistry and Molecular Biology, Volume 9, Sequencing of Proteins and Peptides, G Allen, 2nd revised edition, Elsevier, 1989.

Particularly preferred methods include ARMS™ and RFLP based methods. ARMS™ is an especially preferred method.

Antibodies can be prepared using any suitable method. For example, purified polypeptide may be utilized to prepare specific antibodies. The term "antibodies" is meant to include polyclonal antibodies, monoclonal antibodies, and the various types of antibody constructs such as for example $F(ab')_2$, Fab and single chain Fv. Antibodies are defined to be specifically binding if they bind the allelic variant of OATP-C with a K_a of greater than or equal to about 10^7 M^{-1}. Affinity of binding can be determined using conventional techniques, for example those described by Scatchard et al., *Ann. N.Y. Acad. Sci.*, 51:660 (1949).

Polyclonal antibodies can be readily generated from a variety of sources, for example, horses, cows, goats, sheep, dogs, chickens, rabbits, mice or rats, using procedures that are well-known in the art. In general, antigen is administered to the host animal typically through parenteral injection. The immunogenicity of antigen may be enhanced through the use of an adjuvant, for example, Freund's complete or incomplete adjuvant. Following booster immunizations, small samples of serum are collected and tested for reactivity to antigen. Examples of various assays useful for such determination include those described in:

Antibodies: A Laboratory Manual, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988; as well as procedures such as countercurrent immuno-electrophoresis (CIEP), radioimmunoassay, radioimmunoprecipitation, enzyme-linked immuno-sorbent assays (ELISA), dot blot assays, and sandwich assays, see U.S. Patent Nos. 4,376,110 and 4,486,530.

Monoclonal antibodies may be readily prepared using well-known procedures, see for example, the procedures described in U.S. Patent Nos. RE 32,011, 4,902,614, 4,543,439 and 4,411,993; Monoclonal Antibodies, Hybridomas: *A New Dimension in Biological Analyses*, Plenum Press, Kennett, McKearn, and Bechtol (eds.), (1980).

Monoclonal antibodies can be produced using alternative techniques, such as those described by Alting-Mees et al., "Monoclonal Antibody Expression Libraries: A Rapid Alternative to Hybridomas", *Strategies in Molecular Biology* 3: 1-9 (1990) which is incorporated herein by reference. Similarly, binding partners can be constructed using recombinant DNA techniques to incorporate the variable regions of a gene that encodes a specific binding antibody. Such a technique is described in Larrick et al., *Biotechnology*, 7: 394 (1989).

Once isolated and purified, the antibodies may be used to detect the presence of particular polypeptide variants in a sample using established assay protocols, see for example "A Practical Guide to ELISA" by D. M. Kemeny, Pergamon Press, Oxford, England.

Statins already approved for use in humans include atorvastatin, cerivastatin, fluvastatin, pravastatin and simvastatin. The reader is referred to the following references for further information: Drugs and Therapy Perspectives (12th May 1997), **9**: 1-6; Chong (1997) Pharmacotherapy **17**: 1157-1177; Kellick (1997) Formulary **32**: 352; Kathawala (1991) Medicinal Research Reviews, **11**: 121-146; Jahng (1995) Drugs of the Future **20**: 387-404, and Current Opinion in Lipidology, (1997), **8**, 362 - 368. A preferred statin drug is compound 3a (S-4522) in Watanabe (1997) Bioorganic and Medicinal Chemistry **5**: 437-444; now called rosuvastatin, see Olsson (2001) American Journal of Cardiology, 87, supplement 1, 33-36.

Preferably the statin is rosuvastatin. Preferably the patient is prescribed at least 40mg of rosuvastatin daily, more preferably the patient is prescribed at least 60mg of rosuvastatin daily and especially the patient is prescribed at least 80mg of rosuvastatin daily.

Preferably the patient is additionally tested for presence of valine at position 174 of OATP-C polypeptide whereby presence of both valine and alanine at position 174 indicates heterozygosity at this locus.

Preferably the polymorphism in linkage disequilibrium with alanine174 OATP-C is selected from at least one of:

a) Asp130 OATP-C; or

b) consensus NF1 transcription factor binding sites at positions –26A>G or –118A>C relative to the transcription initiation site (SEQ ID No 2); or

c) -309T>C, -878A>G, -903C>T, -1054G>T, -1215T>A or –1558 T>C, where nucleotide positions are relative to the transcription initiation site (SEQ ID No 2); or

d) polymorphisms in the 3'UTR region of the OATP-C gene selected from T2122G, C2158T, A2525C, and G2651A, where the nucleotide position is relative to the ATG, and the A of the ATG is nucleotide +1 (sequence accession number AF205071 and SEQ ID No 3)

The transcription initiation site is defined in Jung, D. 2001 Journal of Biological Chemistry. 276(40), 37206-37214.

In one embodiment the biological sample is tested for presence of an amino acid at a position of the OATP-C polypeptide through analysis of genetic material encoding the polypeptide.

Another aspect of the invention provides an in vitro method of monitoring a patient for an adverse event related to statin therapy wherein the method comprises testing a biological sample from the patient for a parameter indicative of an adverse event and wherein the patient is selected for such monitoring by a method described herein.

Preferably the patient is OATPC*5 or *15 genotype. As patients carry 2 copies of the OATPC gene they may be homozygous or heterozygous genotype.

According to another aspect of the present invention there is provided a method for the detection of a polymorphism in OATPC in a human, which method comprises determining the sequence of the human at at least one of the following polymorphic positions:

a) consensus NF1 transcription factor binding sites at positions –26A>G or –118A>C relative to the transcription initiation site; or

b) -309T>C, -878A>G, -903C>T, -1054G>T, -1215T>A or –1558 T>C, where nucleotide positions are relative to the transcription initiation site; or

c) polymorphisms in the 3'UTR region of the OATP-C gene selected from T2122G, C2158T, A2525C, and G2651A, where the nucleotide position is relative to the ATG , and the A of the ATG is nucleotide +1 (sequence accession number AF205071 and SEQ ID No 3).

According to another aspect of the present invention there is provided a human OATPC gene or its complementary strand comprising a variant allelic polymorphism at one

or more of positions defined herein or a fragment thereof of at least 20 bases comprising at least one novel polymorphism.

According to another aspect of the present invention there is provided an allele specific primer capable of detecting a OATPC gene polymorphism, preferably at one or more of the positions as defined herein.

An allele specific primer is used, generally together with a constant primer, in an amplification reaction such as a PCR reaction, which provides the discrimination between alleles through selective amplification of one allele at a particular sequence position e.g. as used for ARMS™ assays. The allele specific primer is preferably 17- 50 nucleotides, more preferably about 17-35 nucleotides, more preferably about 17-30 nucleotides.

An allele specific primer preferably corresponds exactly with the allele to be detected but derivatives thereof are also contemplated wherein about 6-8 of the nucleotides at the 3' terminus correspond with the allele to be detected and wherein up to 10, such as up to 8, 6, 4, 2, or 1 of the remaining nucleotides may be varied without significantly affecting the properties of the primer.

Primers may be manufactured using any convenient method of synthesis. Examples of such methods may be found in standard textbooks, for example "Protocols for Oligonucleotides and Analogues; Synthesis and Properties," Methods in Molecular Biology Series; Volume 20; Ed. Sudhir Agrawal, Humana ISBN: 0-89603-247-7; 1993; 1st Edition. If required the primer(s) may be labelled to facilitate detection.

According to another aspect of the present invention there is provided an allele-specific oligonucleotide probe capable of detecting a OATPC gene polymorphism, preferably at one or more of the positions defined herein.

The allele-specific oligonucleotide probe is preferably 17- 50 nucleotides, more preferably about 17-35 nucleotides, more preferably about 17-30 nucleotides.

The design of such probes will be apparent to the molecular biologist of ordinary skill. Such probes are of any convenient length such as up to 50 bases, up to 40 bases, more conveniently up to 30 bases in length, such as for example 8-25 or 8-15 bases in length. In general such probes will comprise base sequences entirely complementary to the corresponding wild type or variant locus in the gene. However, if required one or more mismatches may be introduced, provided that the discriminatory power of the oligonucleotide probe is not unduly affected. The probes of the invention may carry one or more labels to facilitate detection.

According to another aspect of the present invention there is provided an allele specific primer or an allele specific oligonucleotide probe capable of detecting a OATPC gene polymorphism at one of the positions defined herein.

According to another aspect of the present invention there is provided a diagnostic kit comprising an allele specific oligonucleotide probe of the invention and/or an allele-specific primer of the invention.

The diagnostic kits may comprise appropriate packaging and instructions for use in the methods of the invention. Such kits may further comprise appropriate buffer(s) and polymerase(s) such as thermostable polymerases, for example taq polymerase.

According to another aspect of the present invention there is provided a method of treating a patient in need of treatment with a statin in which the method comprises:

i) use of an in vitro diagnostic method to identify a patient potentially requiring a statin dose level above the minimum recommended dose level or to identify a patient in which titration to a statin dose level above the minimum recommended dose level should be monitored and in which the method comprises testing a biological sample from the patient for presence of alanine at position 174 of OATP-C polypeptide and/or a polymorphism in linkage disequilibrium therewith; and

ii) administering an effective amount of the drug.

According to another aspect of the invention there is provided use of a statin in preparation of a medicament for treating a patient with vascular disease or a predisposition thereto wherein the patient is identified by an in vitro diagnostic to identify a patient potentially requiring a statin dose level above the minimum recommended dose level or to identify a patient in which titration to a statin dose level above the minimum recommended dose level should be monitored and in which the method comprises testing a biological sample from the patient for presence of alanine at position 174 of OATP-C polypeptide and/or a polymorphism in linkage disequilibrium therewith.

According to another aspect of the invention there is provided a method of classifying a patient in need of statin therapy comprising testing a biological sample from the patient for presence of alanine at position 174 of OATP-C polypeptide and/or a polymorphism in linkage disequilibrium therewith.

According to another aspect of the invention there is provided a method of identifying a patient on statin therapy that requires adverse event monitoring comprising testing a

biological sample from the patient for presence of alanine at position 174 of OATP-C polypeptide and/or a polymorphism in linkage disequilibrium therewith.

"Adverse event" means the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (eg. nausea, chest pain), signs (eg. tachycardia, enlarged liver) or the abnormal results of an investigation (eg. laboratory findings, electrocardiogram).

"Linkage disequilibrium" means the occurrence of alleles at genetic loci together, more often than would be expected by chance.

"Patient" means a person who is receiving medical treatment.

"Dose" means quantity to be administered at one time, such as a specified amount of medication. For rosuvastatin, the adult starting dose is usually 10mg daily. Higher doses may be required to produce desired lipid profiles in some patients.

"Benefit Risk ratio" means the relation between the risks and benefits of a given treatment or procedure.

An acceptable risk relates to the potential for suffering disease or injury that will be tolerated by an individual in exchange for the benefits of using a substance or process that will cause such disease or injury. Acceptability of risk depends on scientific data, social, economic, and political factors, and on the perceived benefits arising from a chemical or process that creates the risk(s) in question.

According to another aspect of the invention there is provided a method of testing for an adverse event in a patient, the method comprising

(a) identifying a patient who

(i) is in need of treatment with a therapeutic agent that is transported by OATP-C, and

(ii) has (A) an alanine at the amino acid position of OATP-C corresponding to position 174 of SEQ ID NO:1, or (B) a polymorphism in linkage disequilibrium with (A);

(b) providing a biological sample from the patient after the patient undergoes treatment with the therapeutic agent; and

(c) testing the sample for a parameter indicative of an adverse event related to treatment with the therapeutic agent.

According to another aspect of the invention there is provided a method of treatment comprising:

(a) identifying a patient in need of treatment with a therapeutic agent that is transported by OATP-C;

(b) determining whether the patient has either or both of

(i) an alanine at the amino acid position of OATP-C corresponding to position 174 of SEQ ID NO:1, or

(ii) a polymorphism in linkage disequilibrium with (i); and

(c) prescribing an appropriate dosage of the therapeutic agent.

Preferably the method further comprises:

(d) monitoring the patient for an adverse event relating to reduced transport of the therapeutic agent.

According to another aspect of the invention there is provided a method for characterizing the genotype of a human identified as being in need of a treatment with a drug transportable by OATP-C, the method comprising:

(a) providing a nucleic acid sample from the human, wherein the sample comprises a first nucleotide at a position corresponding to position 620 of SEQ ID NO:1;

(b) testing the sample to determine the identity of the first nucleotide;

(c) recording the identity of the first nucleotide in print or in a machine-readable medium; and

(d) communicating the identity of the first nucleotide to the human or to the human's caregiver.

According to another aspect of the invention there is provided a method for evaluating an OATP-C gene in a human, the method comprising:

(a) receiving a request for performing a haplotype analysis of a human from a client, wherein the human is in need of treatment with a therapeutic agent transportable by OATP-C;

(b) accepting a nucleic acid sample of the human;

(c) testing the sample to determine the presence of a variant described herein; and

(d) providing results of the testing to a party,

thereby evaluating the OATP-C gene.

According to another aspect of the invention there is provided a method to assess the pharmacogenetics of a drug, the method comprising:

(a) providing a nucleic acid sample from a human;

(b) determining the presence of a OATP-C variant described herein; and

(c) correlating (i) the identity of the nucleotide with (ii) the human's response following administration of a drug, thereby assessing the pharmacogenetics of the drug.

According to another aspect of the invention there is provided a computer-accessible medium comprising a database that includes a plurality of records, wherein each record associates (a) information that identifies a subject, with (b) information that indicates whether the subject has a variant described herein, and wherein each record further associates (a) with (c) information that identifies the presence or absence of an adverse event in the subject resulting from administration of an OATP-C-transportable drug to the subject.

According to another aspect of the invention there is provided an article of computer-readable medium having instructions encoded thereon, the instructions causing a processor to effect a method comprising:

(a) receiving information that indicates whether a subject has a variant described herein; and

(b) suggesting an appropriate dosage of an OATP-C-transportable agent, wherein the suggestion is based on the information of (a).

Preferably the article is one wherein the suggested dosage is displayed in print or in an electronic format.

The invention will now be illustrated by the following non-limiting Examples in which:

Figure 1 shows the effect of the V174A polymorphism on plasma levels of rosuvastatin. Correlation between genotype, for 3 non-synonymous SNPs in OATP-C, and dose-normalised plasma rosuvastatin values (ng/ml/mg) illustrates that the 174Ala variant is associated with higher plasma concentrations. (WT = wild-type homozygote, HET = heterozygote, VAR = homozygous variant.) None of the 52 subjects analysed were homozygous variant for either the Val174Ala or the Pro155Thr variants. Of the 52 subjects analysed, 42 were recorded as being of Caucasian origin. The other 10 subjects were either Hispanic, Black or Asian.

Figure 2 shows the effect of the OATP-C*15 haplotype on plasma levels of rosuvastatin. Correlation between OATP-C haplotypes and dose-normalised plasma rosuvastatin values (ng/ml/mg) illustrates that the OATP-C*15 haplotype is associated with higher plasma

concentrations. See Table 1 below for a description of amino acid variants for each haplotype. Results for subjects haplotype pairs with n=3 or fewer (*15/*14, *1b/*14, *1b/*15) are not shown.

In Figures 1 and 2, the lower and upper lines of the "box" are the 25th and 75th percentiles of the sample. The distance between the top and bottom of the box is the interquartile range. The line in the middle of the box is the sample median. The "whiskers", extending above and below the box, show the extent of the rest of the sample (unless there are outliers). Assuming no outliers, the maximum of the sample is the top of the upper whisker. The minimum of the sample is the bottom of the lower whisker. By default, an outlier is a value that is more than 1.5 times the interquartile range away from the top or bottom of the box. Individual data points are outliers.

Figure 3 shows the effect of the Val174Ala variant on plasma levels of rosuvastatin in patients treated for 6 weeks with different doses of rosuvastatin. Plasma rosuvastin levels at 6 weeks have been dose normalised for the analysis. Mean plasma rosuvastatin levels were higher in subjects heterozygous for the Val174Ala polymorphism, as compared to homozygous wild-type subjects (Val/Val). The association between the V174A variant allele and plasma rosuvastatin PK levels was most evident at the higher doses of rosuvastatin.
Figure 4 shows that there is a trend for an increase in the mean plasma rosuvastatin concentrations in those subjects who are heterozygous for the SNP az0005537. This SNP is located within a putative NF1 transcription factor binding site at –118 bp upstream of the start of transcription. Data shown is for two independent phase III studies where PK data was collected. Genotype 1 1 is the wild-type homozygous genotype (az0005537 A/A) and genotype 1 2 is the heterozygous genotype (az0005537 A/C).
Figure 5 shows that subjects that have the linked 174A variant and minor C allele at the az0005537 SNP have a tendency for higher mean plasma rosuvastatin concentrations in comparison to subjects with the V174 variant and the major common az0005537 allele (A). Hence the variant az0005537 allele (C) appears to have an additive effect on plasma rosuvastatin levels.

Since alleles of SNP az0005537 are in linkage disequlibrium with those of OATPC V174A, the variant allele at the SNP in the promoter region may increase the expression of the reduced function OATPC allele resulting in increased plasma rosuvastatin levels in subjects which have both of these polymorphic variants. Data shown is combined from 2 phase III clinical studies where PK data was collected. Subjects heterozygous for the V174A

variant have been stratified into two groups based on the genotype for the promoter az0005537 polymorphism. NF1 WT = subjects with A/A wild-type genotype at the az0005537 SNP. NF1 het = subjects with the A/C heterozygous genotype at the AZ0005537 SNP.

Example 1

Polymorphisms in OATP-C affect the *in vivo* disposition of statins in patients

In brief, the OATP-C gene was sequenced in 79 human clinical trial subjects. 52 of these patients had received rosuvastatin for at least 6 weeks for dislipidaemic disease and had plasma PK measurements taken after 6 weeks of treatment. Data for these 52 patients were used to show that some polymorphic variants in OATP-C have a functionally significant effect on plasma levels of statins.

Methodology

The promoter, exons and 3' untranslated regions of OATP-C were fully sequenced by DNA terminator sequencing in DNA collected from 79 subjects in clinical trials. Sequencing traces were used to record the genotypes for known (i.e. available in the literature or SNP databases) SNPs in OATP-C. Some novel SNPs were found in the promoter and 3'UTR region of OATP-C.

Mean dose-normalised plasma rosuvastatin concentrations were determined for the genotypes for each polymorphic variant in the OATP-C gene. OATP-C genotype data for 3 SNPs, namely amino acid position 130 Asparagine → Aspartic acid (Asn130Asp), 155 Proline → Threonine (Pro155Thr) and 174 Valine→Alanine (Val174Ala), was utilised to determine the haplotype pair for each subject. Mean dose-normalised plasma rosuvastatin concentrations were determined for the subjects grouped by haplotype pair.

All consenting subjects treated with rosuvastatin (n=271), from the 2 clinical trials including the original 52 patients, were subsequently genotyped, using TaqMan™, for OATP-C variants. Data for SNPs causing the N130D, P155T and V174A variants were utilised to assign OATPC haplotype pairs to each subject, as previously described.

Results

The sequencing data revealed a number of SNPs not previously reported, 8 in the promoter region (Jung, D. 2001 Journal of Biological Chemistry. 276(40), 37206-37214) and 4 in the 3'UTR region of OATP-C. These SNPs represent another aspect of the invention. These 12 novel SNPs were identified via sequencing the OATP-C gene in 79 subjects. Of

these novel SNPs, 8 SNPs were located in the OATP-C promoter. 2 of these SNPs were located in consensus NF1 transcription factor binding sites at positions –26A>G and –118A>C relative to the transcription initiation site (see SEQ ID NO 2). Other novel upstream SNPs included, -309T>C, -878A>G, -903C>T, -1054G>T, -1215T>A and –1558 T>C, where nucleotide positions are relative to the transcription initiation site as described by Jung et al (SEQ ID NO 2). A further 4 novel SNPs were located in the 3'UTR region of the OATP-C gene, namely T2122G, C2158T, A2525C, and G2651A, where the nucleotide position is relative to the ATG (see SEQ ID NO 3).

OATP-C genotype data for 3 SNPs Asn130Asp, Pro155Thr and Val174Ala was utilised to determine the haplotypes. The package SNPHAP (Clayton, David SNPHAPv0.2 2002 http://www-gene.cimr.cam.ac.uk/clayton/software/snphap.txt) was used for this analysis. The haplotypes were also predicted using the PHASE package (Stephens, M. 2001 American Journal of Human Genetics **68**, 978-989) and were found to give the same predicted haplotypes. The haplotypes were found to be concordant with those reported previously (Tirona 2001). The –118 A>C promoter SNP, at the NF1 binding site, was in strong linkage disequilibrium with the Val174Ala coding variant (delta = 0.3).

Table 1 Common haplotypes in the OATP-C gene

* nomenclature	Amino acid variant(s) on allele	Haplotype Frequency (n=79)
*1a	Asp130 , Pro155 , Val174	57%
*1b	**Asn130** , Pro155 , Val 174	22%
*5	Asp130 , Pro155 , **Ala174**	2%
*14	**Asn130** , **Thr155** , Val174	7.5%
*15	**Asn130** , Pro155 , **Ala174**	11.5%

Table 2 Frequency of the more common non-synonymous OATP-C SNPs (n=79)

Amino Acid	Major allele	Minor allele	SNP	Frequency
130	Asn130	Asp130	A388G	0.41
155	Pro155	Thr 155	C463A	0.08
174	Val174	Ala 174	T521C	0.13

Table 3: Individuals haplotypes for OATP-C

Haplotype Pair	AZ haplotype ID	No of ind (total = 79)	Frequency
*1a/*1a	A	28	35%
*1b/*1b	B	7	9%
*1a/*1b	C	14	18%
*1a/*15	D	13	16%
*1a/*14	E	6	7%
*1b/*15	F	3	4%
*1b/*14	G	3	4%
*1a/*5	H	2	3%
*15/*14	I	3	4%

Table 4

PK GROUP	Val/Val	Val/Ala	Total
HIGH	17	10	27
LOW	22	3	25
Total	39	13	52

Table 4 shows the distribution of genotypes for the V174A variant between subjects classified into 'high' and 'low' PK groups based on the distribution of rosuvastin plasma PK levels in 2 phase III trials. The high and low sub-groups represent those subjects with PK values in the 10^{th} and 90^{th} percentiles compared to the distribution of plasma PK values observed for the complete trial cohort. The Val/Ala heterozygote genotype is more common in those subjects with 'high' plasma PK levels, and more frequent than would be expected by chance based on the population allele frequency of the V174A variant [chi squared p=0.037 when n=52 (all subjects) and p=0.019 when n=42 (Caucasian subjects only)] and [exact test p=0.055 when n=52 (all subjects) and p=0.043 when n=42 (Caucasian subjects only)].

Sequence and genotype data from 79 subjects was used to determine the allele and haplotype frequencies. Plasma rosuvastatin concentrations were only available for 52 of these

79 subjects and hence there were only small numbers of subjects for some of the haplotype pair groups.

The V174A variant usually occurs as the OATPC*15 allele. The V174A variant has also been observed on the OATPC*5 allele in Caucasian populations, but not in Japanese populations. However, statistically significant differences in frequencies of these alleles between populations have not been observed.

Table 5 - Sequences

A) Protein Sequences

Protein sequences determined by translation of cDNA with accession number AF205071

1) Sequence of OATPC protein OATPC*1a (N130 and V174) - SEQ ID NO 1

```
MDQNQHLNKT AEAQPSENKK TRYCNGLKMF LAALSLSFIA KTLGAIIMKS  50
SIIHIERRFE ISSSLVGFID GSFEIGNLLV IVFVSYFGSK LHRPKLIGIG  100
CFIMGIGGVL TALPHFFMGY YRYSKETNIN SSENSTSTLS TCLINQILSL  150
NRASPEIVGK GCLKESGSYM WIYVFMGNML RGIGETPIVP LGLSYIDDFA  200
KEGHSSLYLG ILNAIAMIGP IIGFTLGSLF SKMYVDIGYV DLSTIRITPT  250
DSRWVGAWWL NFLVSGLFSI ISSIPFFFLP QTPNKPQKER KASLSLHVLE  300
TNDEKDQTAN LTNQGKNITK NVTGFFQSFK SILTNPLYVM FVLLTLLQVS  350
SYIGAFTYVF KYVEQQYGQP SSKANILLGV ITIPIFASGM FLGGYIIKKF  400
KLNTVGIAKF SCFTAVMSLS FYLLYFFILC ENKSVAGLTM TYDGNNPVTS  450
HRDVPLSYCN SDCNCDESQW EPVCGNNGIT YISPCLAGCK SSSGNKKPIV  500
FYNCSCLEVT GLQNRNYSAH LGECPRDDAC TRKFYFFVAI QVLNLFFSAL  550
GGTSHVMLIV KIVQPELKSL ALGFHSMVIR ALGGILAPIY FGALIDTTCI  600
KWSTNNCGTR GSCRTYNSTS FSRVYLGLSS MLRVSSLVLY IILIYAMKKK  650
YQEKDINASE NGSVMDEANL ESLNKNKHFV PSAGADSETH C.  692
```

2) Sequence of OATPC protein OATPC*15 (D130 and A174) - SEQ ID NO: 4

```
MDQNQHLNKT AEAQPSENKK TRYCNGLKMF LAALSLSFIA KTLGAIIMKS  50
SIIHIERRFE ISSSLVGFID GSFEIGNLLV IVFVSYFGSK LHRPKLIGIG  100
CFIMGIGGVL TALPHFFMGY YRYSKETNID SSENSTSTLS TCLINQILSL  150
```

NRASPEIVGK GCLKESGSYM WIYAFMGNML RGIGETPIVP LGLSYIDDFA 200
KEGHSSLYLG ILNAIAMIGP IIGFTLGSLF SKMYVDIGYV DLSTIRITPT 250
DSRWVGAWWL NFLVSGLFSI ISSIPFFFLP QTPNKPQKER KASLSLHVLE 300
TNDEKDQTAN LTNQGKNITK NVTGFFQSFK SILTNPLYVM FVLLTLLQVS 350
SYIGAFTYVF KYVEQQYGQP SSKANILLGV ITIPIFASGM FLGGYIIKKF 400
KLNTVGIAKF SCFTAVMSLS FYLLYFFILC ENKSVAGLTM TYDGNNPVTS 450
HRDVPLSYCN SDCNCDESQW EPVCGNNGIT YISPCLAGCK SSSGNKKPIV 500
FYNCSCLEVT GLQNRNYSAH LGECPRDDAC TRKFYFFVAI QVLNLFFSAL 550
GGTSHVMLIV KIVQPELKSL ALGFHSMVIR ALGGILAPIY FGALIDTTCI 600
KWSTNNCGTR GSCRTYNSTS FSRVYLGLSS MLRVSSLVLY IILIYAMKKK 650
YQEKDINASE NGSVMDEANL ESLNKNKHFV PSAGADSETH C. 692

3) Sequence of OATPC protein OATPC*5 (N130 and A174) - SEQ ID NO:5

MDQNQHLNKT AEAQPSENKK TRYCNGLKMF LAALSLSFIA KTLGAIIMKS 50
SIIHIERRFE ISSSLVGFID GSFEIGNLLV IVFVSYFGSK LHRPKLIGIG 100
CFIMGIGGVL TALPHFFMGY YRYSKETNIN SSENSTSTLS TCLINQILSL 150
NRASPEIVGK GCLKESGSYM WIYAFMGNML RGIGETPIVP LGLSYIDDFA 200
KEGHSSLYLG ILNAIAMIGP IIGFTLGSLF SKMYVDIGYV DLSTIRITPT 250
DSRWVGAWWL NFLVSGLFSI ISSIPFFFLP QTPNKPQKER KASLSLHVLE 300
TNDEKDQTAN LTNQGKNITK NVTGFFQSFK SILTNPLYVM FVLLTLLQVS 350
SYIGAFTYVF KYVEQQYGQP SSKANILLGV ITIPIFASGM FLGGYIIKKF 400
KLNTVGIAKF SCFTAVMSLS FYLLYFFILC ENKSVAGLTM TYDGNNPVTS 450
HRDVPLSYCN SDCNCDESQW EPVCGNNGIT YISPCLAGCK SSSGNKKPIV 500
FYNCSCLEVT GLQNRNYSAH LGECPRDDAC TRKFYFFVAI QVLNLFFSAL 550
GGTSHVMLIV KIVQPELKSL ALGFHSMVIR ALGGILAPIY FGALIDTTCI 600
KWSTNNCGTR GSCRTYNSTS FSRVYLGLSS MLRVSSLVLY IILIYAMKKK 650
YQEKDINASE NGSVMDEANL ESLNKNKHFV PSAGADSETH C. 692

B) Sequence of OATPC cDNA (AF205071) for 3'UTR SNPs

Coding region is nucleotides 135 to 2210. Position of polymorphisms downstream from the ATG (upper case) are described where the A of the ATG is +1.

Polymorphisms T2122G, C2158T, A2525C, G2651A are underlined
SEQ ID NO: 3

cggacgcgtg ggcggacgcg tgggtcgccc acgcgtccga cttgttgcag 50
ttgctgtagg attctaaatc caggtgattg tttcaaactg agcatcaaca 100
acaaaaacat ttgtatgata tctatatttc aatcATGgac caaaatcaac 150
atttgaataa aacagcagag gcacaacctt cagagaataa gaaaacaaga 200
tactgcaatg gattgaagat gttcttggca gctctgtcac tcagctttat 250
tgctaagaca ctaggtgcaa ttattatgaa aagttccatc attcatatag 300
aacggagatt tgagatatcc tcttctcttg ttggttttat tgacggaagc 350
tttgaaattg gaaatttgct tgtgattgta tttgtgagtt actttggatc 400
caaactacat agaccaaagt taattggaat cggttgtttc attatgggaa 450
ttggaggtgt tttgactgct ttgccacatt tcttcatggg atattacagg 500
tattctaaag aaactaatat cgattcatca gaaaattcaa catcgacctt 550
atccacttgt ttaattaatc aaattttatc actcaataga gcatcacctg 600
agatagtggg aaaaggttgt ttaaaggaat ctgggtcata catgtggata 650
tatgtgttca tgggtaatat gcttcgtgga ataggggaga ctcccatagt 700
accattgggg ctttcttaca ttgatgattt cgctaaagaa ggacattctt 750
ctttgtattt aggtatattg aatgcaatag caatgattgg tccaatcatt 800
ggctttaccc tgggatctct gttttctaaa atgtacgtgg atattggata 850
tgtagatcta agcactatca ggataactcc tactgattct cgatgggttg 900
gagcttggtg gcttaatttc cttgtgtctg gactattctc cattatttct 950
tccataccat tctttttctt gccccaaact ccaaataaac cacaaaaaga 1000
aagaaaagct tcactgtctt tgcatgtgct ggaaacaaat gatgaaaagg 1050
atcaaacagc taatttgacc aatcaaggaa aaaatattac caaaaatgtg 1100
actggttttt tccagtcttt taaaagcatc cttactaatc ccctgtatgt 1150
tatgtttgtg cttttgacgt tgttacaagt aagcagctat attggtgctt 1200
ttacttatgt cttcaaatac gtagagcaac agtatggtca gccttcatct 1250
aaggctaaca tcttattggg agtcataacc atacctattt ttgcaagtgg 1300
aatgttttta ggaggatata tcattaaaaa attcaaactg aacaccgttg 1350
gaattgccaa attctcatgt tttactgctg tgatgtcatt gtccttttac 1400
ctattatatt ttttcatact ctgtgaaaac aaatcagttg ccggactaac 1450
catgacctat gatggaaata atccagtgac atctcataga gatgtaccac 1500
tttcttattg caactcagac tgcaattgtg atgaaagtca atgggaacca 1550
gtctgtggaa acaatggaat aacttacatc tcaccctgtc tagcaggttg 1600
caaatcttca agtggcaata aaaagcctat agtgttttac aactgcagtt 1650
gtttggaagt aactggtctc cagaacagaa attactcagc ccatttgggt 1700
gaatgcccaa gagatgatgc ttgtacaagg aaattttact tttttgttgc 1750
aatacaagtc ttgaatttat ttttctctgc acttggaggc acctcacatg 1800

tcatgctgat tgttaaaatt gttcaacctg aattgaaatc acttgcactg 1850
ggtttccact caatggttat acgagcacta ggaggaattc tagctccaat 1900
atattttggg gctctgattg atacaacgtg tataaagtgg tccaccaaca 1950
actgtggcac acgtgggtca tgtaggacat ataattccac atcattttca 2000
agggtctact tgggcttgtc ttcaatgtta agagtctcat cacttgtttt 2050
atatattata ttaatttatg ccatgaagaa aaaatatcaa gagaaagata 2100
tcaatgcatc agaaaatgga agtgtcatgg atgaagcaaa cttagaatcc 2150
ttaaataaaa ataaacattt tgtcccttct gctggggcag atagtgaaac 2200
acattgttaa ggggagaaaa aaagccactt ctgcttctgt gtttccaaac 2250
agcattgcat tgattcagta agatgttatt tttgaggagt tcctggtcct 2300
ttcactaaga atttccacat cttttatggt ggaagtataa ataagcctat 2350
gaacttataa taaaacaaac tgtaggtaga aaaaatgaga gtactcattg 2400
ttacattata gctacatatt tgtggttaag gttagactat atgatccata 2450
caaattaaag tgagagacat ggttactgtg taataaaaga aaaaatactt 2500
gttcaggtaa ttctaattct taataaaaca aatgagtatc atacaggtag 2550
aggttaaaaa ggaggagcta gattcatatc ctaagtaaag agaaatgcct 2600
agtgtctatt ttattaaaca aacaaacaca gagtttgaac tataatacta 2650
aggcctgaag tctagcttgg atatatgcta caataatatc tgttactcac 2700
ataaaattat atatttcaca gactttatca atgtataatt aacaattatc 2750
ttgtttaagt aaatttagaa tacatttaag tattgtggaa gaaataaaga 2800
cattccaata tttgcaaaaa aaaaaaaaaa 2830

C) Sequence of OATPC promoter region from AC022335

-26A>G or -118A>C, and -309T>C, -878A>G, -903C>T, -1054G>T, -1215T>A or -1558 T>C, where nucleotide positions are relative to the transcription initiation site (as defined by Jung et al) and as according to the following promoter and 5' flanking sequence (from AC022335).

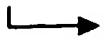
The start of transcription is annotated with an arrow.
SNPs are marked in upper case.

SEQ ID NO: 2

atctcagaga ttttatttgt attcatttaa tataaattaa ctgctctaaa -1951

atttataata tgcaaatatc atacaattaa tctaattagg tgttgaatct -1901
ataatgtgcc aggcattatg taaggcactt tacatacact aaatctttat -1851
tccaaatata gacttcttac tttatagatg agtgcactga tgctcagaaa -1801
tggtaaataa cctactgatg tttatactgc tggcaggtag cagagacata -1751
tcggcattta agtctttcag acttcaaagg ccatgatatt tcatcagagc -1701
tgtgatagcc gttcctgaaa aaaatatcag ctgattcttt aaatcaattt -1651
ttgtcatcta actgatgcgt ggctgttagc ataatattga tcttgaaaga -1601
tgttttgcaa catctttccc ctggtgtact cttgtttttc caTgatccca -1551
caaaatgagc agtctaatta tttacacaat taggaagaga aaaggggcac -1501
agagaatgct ctttgacctc tgaaaatatt ggagaatttt acaactggca -1451
cctttagctc aggattataa aggttgttag ttagtttgta ctgttttatc -1401
ttcattgtat ataatatata tattagtctc caaacatgtt gatgtgtttt -1351
caatgaaatg gatgtctgag gagaaaacca ttagcctgag aaaacccaaa -1301
ctgtattccc attgtgaata aaaggaagtc cataaaaatg atggaaaatg -1251
ttctgcattc ctgttatgat atcaaaatct ggcagTacat gaaaattttt -1201
caaagtgctt atttaacagg cataatcttt ggtctcctga gccagaatct -1151
gctgggtatg ggactggatt gctattttga caactcgcca gtagattctt -1101
actcagcaga gtatttggaa gccttactct aatattttgg ccttggTtct -1051
acatttctca gttctgcaca gtcattcttc ccctctacac tactctttag -1001
tttgtctcat gattccaata ctctcaataa ttaaccaaga atagaactaa -951
tcaatcagat aactgtggca cagacatcaa atacattttg ctgcaacCat -900
atcaacaaat gtcccatgaa tgAtaagggg taaccatatt ctcatatatg -851
catcctcaca ttaccacata tatatatgtg catatgtgta tacaggtaaa -800
agtgtgtata tatgtataca tgtatgtttg tgtgtatata catacatata -751
tcttcacact tttctgaaat atatatattt atgtgagaga agggtctgta -700
ctttatttca gaagagagct taatgtccaa ggtataattg agagtctaaa -651
atgtttgagt tattgaatta attaaacttc atctctactc aagaaaactt -600
ttaactgagt taagctcttc ctttctccac aagtcaagtc aataaaagga -551
aactgtgata ttaataattc tttcctgttt tgatgtaaag aatctatcgc -501
ataaagcagt cttaattttc atcattcaga aaaatggtct tgcagttaat -451
tgggactctc ttattccagg tggtatctcc agtctccata cataccacgt -401
tagaaccata cttatgtacc aagcaaagag ggtatatttt aatttttaaa -351
tgccaatgta acctgtaggc atatttttta tttgtcttaa aTtatttcct -301
atttggaagt tttaaatacc tggaataatt tattgtactc atatttttaa -251
agaaaaaaat cttatgccac caacttaatt gaataaacaa gtaaaagcca -201
ttcccaaaag taaggtttac ttgttaagat taacaaaaaa taatgtgaga -151
attctgagaa atataatctt taaatattgg caActggagt gaactcttaa -101

aactaactag gttttatatg tttgactaga gcaatgacat aataaggtgg -51
ttaatcatca ctggacttgt tttcAaaaag ccaactactt taagaggaat -1
aaagggtgga cttgttgcag ttgctgtagg attctaaatc caggtaagaa



Conclusions

Evidence of an *in vivo* genotype-phenotype relationship has been determined between OATP-C variants and the pharmacokinetic profile of statins, a common class of drugs used in the treatment of hypercholesterolaemia / dyslipidaemia. The observation of higher plasma concentrations of rosuvastatin in patients with the Ala174 OATP-C variant indicates that transport of rosuvastatin by the Ala174 variant is lower than that of the Val174 OATP-C variant. The Ala174 variant thus causes reduced uptake of statins in to the liver and consequent increased plasma levels. Plasma drug concentration is a factor in altering the benefit-risk ratio of statin therapy. OATP-C variants N130D and P155T do not appear to affect the pharmacokinetic disposition of rosuvastatin.

The genotype-phenotype correlation between OATP-C variants and *in vivo* plasma levels of statins may be utilised to optimise the statin dose, appropriate for each individual, via a diagnostic assay for the SNP or protein variant. Optimisation of the plasma level will be important in subjects that require high doses of statins for adequate lowering of cholesterol levels to the desired threshold.

Evidence that polymorphisms in OATP-C affect the *in vivo* disposition of statins indicates that OATP-C variants may affect the clinical response to statins and other clinically relevant drugs that are transported by OATP-C. Correlation of polymorphisms in OATP-C with end of treatment dose-normalised plasma rosuvastatin concentrations, determined in subjects treated for 6 weeks with different doses of rosuvastatin, have shown that OATP-C variants have a functional effect on the *in vivo* pharmacokinetic disposition of rosuvastatin. Subjects heterozygous for the Val174Ala variant have increased mean plasma concentrations of rosuvastatin as compared to subjects homozygous for the wild-type Val174 variant at amino acid position 174. Subjects with a single copy of the OATP-C*15 allele (heterozygous for the Val174Ala and Asn130Asp variants) were found to have higher mean plasma concentrations than subjects with the OATP-C*1a, OATP-C*1b, and OATP-C*14 haplotypes. The observation of higher concentrations of rosuvastatin in subjects with the

Ala174 OATP-C variant indicates that transport by the Ala174 variant is lower than that of the Val174 OATP-C variant. The Ala174 variant causes reduced uptake of statins into the liver and has an impact on the clinical response to statins. OATP-C variants affecting the pharmacokinetic profile of statins may be associated with a decreased benefit-risk ratio of statin therapy as a result of the high concentrations of statins in the circulation. The genotype-phenotype correlation between OATP-C variants and *in vivo* plasma levels of statins may be utilised to optimise the statin dose, appropriate for each individual, via a diagnostic assay for the SNP or protein variant. Optimisation of the plasma level will be important in subjects that require high doses of statins for adequate lowering of cholesterol levels to the desired threshold.

Example 2

Evidence for functional significance of NF1 SNP (az0005537)

Figure 4 shows that there is a trend for an increase in the mean plasma rosuvastatin concentrations in those subjects who are heterozygous for the SNP az0005537. This SNP is located within a putative NF1 transcription factor binding site at –118 bp upstream of the start of transcription.

Figure 5 shows that subjects that have the linked 174A variant and minor C allele at the az0005537 SNP have a tendency for higher mean plasma rosuvastatin concentrations in comparison to subjects with the V174 variant but the major common az0005537 allele (A). Hence the variant az0005537 allele (C) appears to have an additive effect on plasma rosuvastatin levels.

Since alleles of SNP az0005537 are in linkage disequlibrium with those of OATPC V174A, the variant allele at the SNP in the promoter region may increase the expression of the reduced function OATPC allele resulting in increased plasma rosuvastatin levels in subjects which have both of these polymorphic variants.

When considering V174A alone with the full cohort of samples (n= 267), V174A WT compared to V174A heterozygote has a t-test value of p=0.055. However, if V174V WT vs V174A&NF1 (az0005537) compound heterozygote is considered, then the t-test value is p=0.032, which is statistically significant. This suggests that the OATPC NF1 SNP may also be a determinant of the pharmacokinetic disposition of rosuvastatin. Preliminary in vitro functional data supports the hypothesis that the variant C az0005537 allele is associated with increased expression. The promoter polymorphism may drive differential allelic expression with greater expression of the reduced function OATPC allele.

Example 3

Linkage Disequilibrium

The polymorphism -118A>C or –1558T>C of SEQ ID NO:2 were analysed as set out below.

Alleles of polymorphisms at -118 and -1558 are in significant linkage disequilibrium with the alanine allele at position 174 of SEQ ID NO:1 (p=0.009 and 0.025respectively, analysed by the ASSOCIATE program, see Ott J (1999) Analysis of human genetic linkage, 3rd edition. Johns Hopkins University Press, Baltimore).